

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (Currently Amended): A process for producing para-hydroxystyrene comprising:

- a) providing an enzyme source having para-hydroxycinnamic acid decarboxylase activity, ~~[[said]]~~wherein the enzyme source ~~comprising—comprises~~ a polypeptide having the amino acid sequence as set forth in SEQ ID NO:4, and wherein the enzyme source is selected from the group consisting of cell-free extract, partially purified enzyme, and purified enzyme;
- b) contacting ~~[[said]]~~the enzyme source with para-hydroxycinnamic acid in a biphasic reaction medium comprising an aqueous phase and an extractant, ~~[[said]]~~wherein the extractant ~~[[being]]~~is a water-immiscible organic solvent selected from the group consisting of toluene, methyl decanoate, 2-undecanone, dichloromethane, hexane, 2-decanol, 4-decanol, 3-decanone, 4-decanone, 1-nonanol, 2-nonanol, 2-heptanol and mixtures thereof, to form para-hydroxystyrene which is extracted into the extractant of the biphasic reaction medium;
- c) separating the extractant containing the para-hydroxystyrene after step b) from the aqueous phase; and
- d) optionally~~[[;]]~~ recovering the ~~para-hydroxystyrene~~para-hydroxystyrene from the extractant.

Claim 2 (Canceled)

Claim 3 (Canceled)

Claim 4 (Canceled)

Claim 5 (Canceled)

Claim 6 (Canceled)

Claim 7 (Canceled)

Claim 8 (Original): A process according to Claim 1 wherein the enzyme source is immobilized.

Claim 9 (Original): A process according to Claim 1 wherein the extractant is present in the biphasic reaction medium in an amount from about 5% to about 70% by volume.

Claim 10 (Original): A process according to Claim 1 wherein the extractant is present in the biphasic reaction medium in an amount from about 20% to about 50% by volume.

Claim 11 (Original): A process according to Claim 1 wherein the extractant is separated from the aqueous phase by use of a gravity settler, a centrifuge, or a hydrocyclone.

Claim 12 (Original): A process according to Claim 1 wherein the enzyme source is recovered from the aqueous phase of the biphasic reaction medium after the separating of step (c) for reuse.

Claim 13 (Original): A process according to Claim 12 wherein the enzyme source is recovered from the aqueous phase using a method selected from the group consisting of filtration, ultrafiltration, nanofiltration, and centrifugation.

Claim 14 (Original): A process according to Claim 1 wherein the recovering of step (d) is accomplished by means selected from the group consisting of evaporation, distillation, adsorption by resins, and adsorption by molecular sieves.

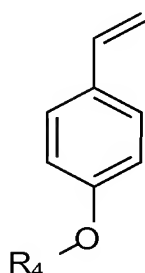
Claim 15 (Original): A process according to Claim 1 wherein after step (d), the extractant is optionally added back to the biphasic reaction medium.

Claim 16 (Original): A process according to Claim 1 wherein the aqueous phase after step (c) is optionally added back to the biphasic reaction medium.

Claim 17 (Currently Amended): A process ~~according to Claim 1 wherein the para-hydroxystyrene is chemically derivatized in the extractant to form a derivatized compound for producing a derivatized compound of para-hydroxystyrene comprising:~~

- a) providing an enzyme source having para-hydroxycinnamic acid decarboxylase activity, wherein the enzyme source comprises a polypeptide having the amino acid sequence as set forth in SEQ ID NO:4, and wherein the enzyme source is selected from the group consisting of cell-free extract, partially purified enzyme, and purified enzyme;
- b) contacting the enzyme source with para-hydroxycinnamic acid in a biphasic reaction medium comprising an aqueous phase and an extractant, wherein the extractant is a water-immiscible organic solvent selected from the group consisting of toluene, methyl decanoate, 2-undecanone, dichloromethane, hexane, 2-decanol, 4-decanol, 3-decanone, 4-decanone, 1-nonanol, 2-nonanol, 2-heptanol and mixtures thereof, to form para-hydroxystyrene which is extracted into the extractant of the biphasic reaction medium;
- c) separating the extractant containing the para-hydroxystyrene after step b) from the aqueous phase; and
- d) chemically derivatizing the extractant containing the para-hydroxystyrene after step c) to form a derivatized compound of para-hydroxystyrene.

Claim 18 (Original): A process according to Claim 17 wherein the derivatized compound is defined by the general formula:



wherein R4 is selected from the group consisting of: methyl, t-butyl, alkyl, silyl ethers, allyl, t-butoxy carbonyl, hydroxyethoxy, acetoxy, formate, glycidyl, benzoate, phenylcarbonate, tetrahydropyran, benzyl, and poly(ethylene oxide).

Claim 19 (Original): A process according to Claim 18 wherein the derivatized compound is para-acetoxystyrene.

Claim 20 (Currently Amended): A process for producing para-hydroxystyrene comprising:

- a) providing a production host which produces para-hydroxycinnamic acid;
- b) growing the production host in a fermentation medium wherein the production host produces para-hydroxycinnamic acid into the fermentation medium;
- c) contacting the fermentation medium from step (b) with an enzyme source having para-hydroxycinnamic acid decarboxylase activity, ~~[[said]]~~ wherein the enzyme source ~~comprising~~ comprises a polypeptide having the amino acid sequence as set forth in SEQ ID NO:4 and the enzyme source is selected from the group consisting of cell-free extract, partially purified enzyme, and purified enzyme, in a biphasic reaction medium comprising the fermentation medium and an extractant, ~~[[said]]~~ and wherein the extractant ~~[[being]]~~ is a water-immiscible organic solvent selected from the group consisting of toluene, methyl decanoate, 2-undecanone, dichloromethane, hexane, 2-decanol, 4-decanol, 3-decanone, 4-decanone, 1-nonanol, 2-nonanol, 2-heptanol and mixtures thereof, to form para-hydroxystyrene~~[[5]]~~ which is extracted into the extractant of the biphasic reaction medium;
- d) separating the extractant containing the para-hydroxystyrene after step c) from the fermentation medium; and
- e) optionally recovering the para-hydroxystyrene from the extractant.

Claim 21 (Original): A process according to Claim 20 wherein the production host and insoluble materials are removed from the fermentation medium prior to the contacting of step (c).

Claim 22 (Original): A process according to Claim 21 wherein the production host and insoluble materials are removed from the fermentation medium by filtration or centrifugation.

Claim 23 (Original): A process according to Claim 20 wherein the production host is selected from the group consisting of *Escherichia*, *Methylosinus*, *Methylomonas*, *Pseudomonas*, *Streptomyces*, *Corynebacterium*, and *Rhodobacter*.

Claim 24 (Canceled)

Claim 25 (Canceled)

Claim 26 (Canceled)

Claim 27 (Canceled)

Claim 28 (Canceled)

Claim 29 (Canceled)

Claim 30 (Original): A process according to Claim 20 wherein the enzyme source is immobilized.

Claim 31 (Original): A process according to Claim 20 wherein the extractant is present in the biphasic reaction medium in an amount from about 5% to about 70% by volume.

Claim 32 (Original): A process according to Claim 20 wherein the extractant is present in the biphasic reaction medium in an amount from about 20% to about 50% by volume.

Claim 33 (Original): A process according to Claim 20 wherein the extractant is separated from the fermentation medium by use of a gravity settler, a centrifuge, or a hydrocyclone.

Claim 34 (Original): A process according to Claim 20 wherein the enzyme source is recovered from the fermentation medium after the separating of step (d) for reuse.

Claim 35 (Currently Amended): A process according to Claim ~~[[35]]~~34 wherein the enzyme source is recovered from the fermentation medium using a method selected from the group consisting of filtration, ultrafiltration, nanofiltration, and centrifugation.

Claim 36 (Original): A process according to Claim 20 wherein the recovering of step (e) is accomplished by means selected from the group consisting of evaporation, distillation, adsorption by resins, and adsorption by molecular sieves.

Claim 37 (Original): A process according to Claim 20 wherein after step (e), the extractant is optionally added back to the biphasic reaction medium.

Claim 38 (Currently Amended): A process according to Claim 20 wherein the fermentation medium after ~~step (e)~~ step (d) is optionally added back to the biphasic reaction medium.

Claim 39 (Currently Amended): ~~A process according to Claim 20 wherein the para-hydroxystyrene is chemically derivatized in the extractant to form a derivatized compound for~~ producing a derivatized compound of para-hydroxystyrene comprising:

- a) providing a production host which produces para-hydroxycinnamic acid;
- b) growing the production host in a fermentation medium wherein the production host produces para-hydroxycinnamic acid into the fermentation medium;
- c) contacting the fermentation medium from step (b) with an enzyme source having para-hydroxycinnamic acid decarboxylase activity, wherein the enzyme source comprises a polypeptide having the amino acid sequence as set forth in SEQ ID NO:4 and the enzyme source is selected from the group consisting of cell-free extract, partially purified enzyme, and purified enzyme, in a biphasic reaction medium comprising the fermentation medium and an extractant, wherein the extractant is a water-immiscible organic solvent selected from the group consisting of toluene, methyl decanoate, 2-undecanone, dichloromethane, hexane, 2-decanol, 4-decanol, 3-decanone, 4-decanone, 1-nonanol, 2-nonanol, 2-heptanol and mixtures thereof, to form para-hydroxystyrene which is extracted into the extractant of the biphasic reaction medium;
- d) separating the extractant containing the para-hydroxystyrene after step c) from the fermentation medium; and
- e) chemically derivatizing the para-hydroxystyrene and the extractant after step d) to form a derivatized compound of para-hydroxystyrene.

Claim 40 (Currently Amended): A process according to Claim ~~[[40]]~~39 wherein the derivatized compound is para-acetoxystyrene.